

DATE: 07/10/2002

PATENT APPLICATION: US/09/975,132A

TIME: 10:51:53

Input Set : A:\GC636-2-SEQLIST.TXT

Output Set: N:\CRF3\07102002\I975132A.raw

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4 <110> APPLICANT: Kolkman, Marc
 6 <120> TITLE OF INVENTION: Enhanced Secretion of a Polypeptide by a
        Microorganism
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12 <141> CURRENT FILING DATE: 2001-10-09
14 <150> PRIOR APPLICATION NUMBER: US 60/239,531
15 <151> PRIOR FILING DATE: 2000-10-10
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PATENT APPLICATION: US/09/975,132A

Input Set : A:\GC636-2-SEQLIST.TXT

Output Set: N:\CRF3\07102002\1975132A.raw

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Output Set: N:\CRF3\07102002\1975132A.raw

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| 298  | <213>   | ORGAN   | ISM:  | Bac   | illus  | s sul  | otil:  | is                                     |   |                                       |  |                                    |   |  |  |   |   |     |
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| 300  | <400>   | SEQUE   | NCE:  | 25  |  |  |  |  | Leu   | Gln                                   | Leu  | Leu                                | Val   | Arq  | Pro  |   |   |     |
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| 300<br>301<br>302<br>303<br>304<br>305<br>306<br>307<br>308  | <pre>&lt;400&gt; Met Se 1 Gly Le Val As Pro Pr 50</pre>   | SEQUE<br>er Arg<br>eu Gln<br>en Cys<br>35<br>to Leu   | NCE:<br>Leu<br>Ala<br>20<br>Ser<br>Pro  | 25<br>Pro<br>5<br>Pro<br>Asn<br>Leu   | Val<br>Met<br>Met<br>Leu                                   | Leu<br>Thr<br>Ile<br>Asp<br>55               | Leu<br>Gln<br>Asp<br>40<br>Phe               | Leu<br>Thr<br>25<br>Glu<br>Asn         | 10<br>Thr<br>Ile<br>Asn                                   | Pro<br>Ile<br>Leu                     | Leu<br>Thr<br>Asn<br>60                      | Lys<br>His<br>45<br>Gly            | Thr<br>30<br>Leu<br>Glu                             | 15<br>Ser<br>Lys<br>Asp                            | Trp<br>Gln<br>Gln                            |   |   |     |
| 300<br>301<br>302<br>303<br>304<br>305<br>306<br>307<br>308<br>309   | <pre>&lt;400&gt; Met Se 1 Gly Le Val As Pro Pr 50 Asp II</pre>  | SEQUE<br>er Arg<br>eu Gln<br>en Cys<br>35<br>to Leu   | NCE:<br>Leu<br>Ala<br>20<br>Ser<br>Pro  | 25<br>Pro<br>5<br>Pro<br>Asn<br>Leu   | Val<br>Met<br>Met<br>Leu<br>Asn                            | Leu<br>Thr<br>Ile<br>Asp<br>55               | Leu<br>Gln<br>Asp<br>40<br>Phe               | Leu<br>Thr<br>25<br>Glu<br>Asn         | 10<br>Thr<br>Ile<br>Asn                                   | Pro<br>Ile<br>Leu<br>Pro              | Leu<br>Thr<br>Asn<br>60                      | Lys<br>His<br>45<br>Gly            | Thr<br>30<br>Leu<br>Glu                             | 15<br>Ser<br>Lys<br>Asp                            | Trp<br>Gln<br>Gln<br>Phe                     |   |   |     |
| 300<br>301<br>302<br>303<br>304<br>305<br>306<br>307<br>308<br>309<br>310  | <pre>&lt;400&gt; Met Se 1 Gly Le Val As Pro Pr 50 Asp Il 65</pre>   | SEQUE<br>r Arg<br>u Gln<br>n Cys<br>35<br>o Leu<br>e Leu  | NCE:<br>Leu<br>Ala<br>20<br>Ser<br>Pro<br>Met   | 25<br>Pro<br>5<br>Pro<br>Asn<br>Leu<br>Glu  | Val<br>Met<br>Met<br>Leu<br>Asn<br>70                      | Leu<br>Thr<br>Ile<br>Asp<br>55<br>Asn        | Leu<br>Gln<br>Asp<br>40<br>Phe<br>Leu        | Leu<br>Thr<br>25<br>Glu<br>Asn<br>Arg  | 10<br>Thr<br>Ile<br>Asn                                   | Pro<br>Ile<br>Leu<br>Pro<br>75        | Leu<br>Thr<br>Asn<br>60<br>Asn               | Lys<br>His<br>45<br>Gly<br>Leu     | Thr<br>30<br>Leu<br>Glu                             | 15<br>Ser<br>Lys<br>Asp                            | Trp<br>Gln<br>Gln<br>Phe<br>80               |   |   |     |
| 300<br>301<br>302<br>303<br>304<br>305<br>306<br>307<br>308<br>309<br>310<br>311   | <pre>&lt;400&gt; Met Se 1 Gly Le Val As Pro Pr 50 Asp II</pre>  | SEQUE<br>r Arg<br>u Gln<br>n Cys<br>35<br>o Leu<br>e Leu  | NCE:<br>Leu<br>Ala<br>20<br>Ser<br>Pro<br>Met   | 25<br>Pro<br>5<br>Pro<br>Asn<br>Leu<br>Glu<br>Lys                                   | Val<br>Met<br>Met<br>Leu<br>Asn<br>70                      | Leu<br>Thr<br>Ile<br>Asp<br>55<br>Asn        | Leu<br>Gln<br>Asp<br>40<br>Phe<br>Leu        | Leu<br>Thr<br>25<br>Glu<br>Asn<br>Arg  | 10<br>Thr<br>Ile<br>Asn<br>Arg                            | Pro<br>Ile<br>Leu<br>Pro<br>75        | Leu<br>Thr<br>Asn<br>60<br>Asn               | Lys<br>His<br>45<br>Gly<br>Leu     | Thr<br>30<br>Leu<br>Glu                             | 15<br>Ser<br>Lys<br>Asp<br>Ala<br>Ser              | Trp<br>Gln<br>Gln<br>Phe<br>80               |   |   |     |
| 300<br>301<br>302<br>303<br>304<br>305<br>306<br>307<br>308<br>309<br>310<br>311<br>312  | <pre>&lt;400&gt; Met Se 1 Gly Le Val As Pro Pr 50 Asp Il 65 Asn Ar</pre>                                    | SEQUE<br>r Arg<br>u Gln<br>n Cys<br>35<br>o Leu<br>e Leu  | NCE:<br>Leu<br>Ala<br>20<br>Ser<br>Pro<br>Met   | 25<br>Pro<br>5<br>Pro<br>Asn<br>Leu<br>Glu<br>Lys<br>85                             | Val<br>Met<br>Leu<br>Asn<br>70<br>Ser                      | Leu<br>Thr<br>Ile<br>Asp<br>55<br>Asn<br>Leu | Leu<br>Gln<br>Asp<br>40<br>Phe<br>Leu<br>Gln | Leu<br>Thr<br>25<br>Glu<br>Asn<br>Arg  | 10<br>Thr<br>Ile<br>Asn<br>Arg<br>Ala<br>90               | Pro<br>Ile<br>Leu<br>Pro<br>75<br>Ser | Leu<br>Thr<br>Asn<br>60<br>Asn               | Lys His 45 Gly Leu Ile             | Thr<br>30<br>Leu<br>Glu<br>Glu                      | 15<br>Ser<br>Lys<br>Asp<br>Ala<br>Ser<br>95        | Trp<br>Gln<br>Gln<br>Phe<br>80<br>Ile        |   |   |     |
| 300<br>301<br>302<br>303<br>304<br>305<br>306<br>307<br>308<br>309<br>310<br>311<br>312<br>313   | <pre>&lt;400&gt; Met Se 1 Gly Le Val As Pro Pr 50 Asp Il 65</pre>   | SEQUE<br>r Arg<br>u Gln<br>n Cys<br>35<br>o Leu<br>e Leu  | NCE:<br>Leu<br>Ala<br>20<br>Ser<br>Pro<br>Met<br>Val<br>Leu                             | 25<br>Pro<br>5<br>Pro<br>Asn<br>Leu<br>Glu<br>Lys<br>85                             | Val<br>Met<br>Leu<br>Asn<br>70<br>Ser                      | Leu<br>Thr<br>Ile<br>Asp<br>55<br>Asn<br>Leu | Leu<br>Gln<br>Asp<br>40<br>Phe<br>Leu<br>Gln | Leu Thr 25 Glu Asn Arg Asn Pro         | 10<br>Thr<br>Ile<br>Asn<br>Arg<br>Ala<br>90               | Pro<br>Ile<br>Leu<br>Pro<br>75<br>Ser | Leu<br>Thr<br>Asn<br>60<br>Asn               | Lys His 45 Gly Leu Ile             | Thr<br>30<br>Leu<br>Glu<br>Glu<br>Glu               | 15<br>Ser<br>Lys<br>Asp<br>Ala<br>Ser<br>95        | Trp<br>Gln<br>Gln<br>Phe<br>80<br>Ile        |   |   |     |
| 300<br>301<br>302<br>303<br>304<br>305<br>306<br>307<br>308<br>309<br>310<br>311<br>312<br>313<br>314  | <pre>&lt;400&gt; Met Se 1 Gly Le Val As Pro Pr 50 Asp Il 65 Asn Ar Leu Ly</pre>                             | SEQUE<br>T Arg<br>u Gln<br>n Cys<br>35<br>to Leu<br>e Leu<br>g Ala  | NCE:<br>Leu<br>Ala<br>20<br>Ser<br>Pro<br>Met<br>Val<br>Leu<br>100                      | Pro<br>5<br>Pro<br>Asn<br>Leu<br>Glu<br>Lys<br>85<br>Leu                            | Val<br>Met<br>Leu<br>Asn<br>70<br>Ser                      | Leu Thr Ile Asp 55 Asn Leu Cys               | Leu Gln Asp 40 Phe Leu Gln Leu               | Leu Thr 25 Glu Asn Arg Asn Pro 105     | 10<br>Thr<br>Ile<br>Asn<br>Arg<br>Ala<br>90<br>Leu        | Pro Ile Leu Pro 75 Ser Ala            | Leu<br>Thr<br>Asn<br>60<br>Asn<br>Ala<br>Thr | Lys His 45 Gly Leu Ile Ala         | Thr<br>30<br>Leu<br>Glu<br>Glu<br>Glu<br>Ala<br>110 | 15<br>Ser<br>Lys<br>Asp<br>Ala<br>Ser<br>95<br>Pro | Trp Gln Gln Phe 80 Ile Thr                   |   |   |     |
| 300<br>301<br>302<br>303<br>304<br>305<br>306<br>307<br>308<br>309<br>310<br>311<br>312<br>313<br>314<br>315   | <pre>&lt;400&gt; Met Se 1 Gly Le Val As Pro Pr 50 Asp Il 65 Asn Ar</pre>                                    | SEQUE<br>T Arg  | NCE:<br>Leu<br>Ala<br>20<br>Ser<br>Pro<br>Met<br>Val<br>Leu<br>100<br>Ile               | Pro<br>5<br>Pro<br>Asn<br>Leu<br>Glu<br>Lys<br>85<br>Leu                            | Val<br>Met<br>Leu<br>Asn<br>70<br>Ser                      | Leu Thr Ile Asp 55 Asn Leu Cys               | Leu Gln Asp 40 Phe Leu Gln Leu Asp           | Leu Thr 25 Glu Asn Arg Asn Pro 105     | 10<br>Thr<br>Ile<br>Asn<br>Arg<br>Ala<br>90<br>Leu        | Pro Ile Leu Pro 75 Ser Ala            | Leu<br>Thr<br>Asn<br>60<br>Asn<br>Ala<br>Thr | Lys His 45 Gly Leu Ile Ala Glu     | Thr<br>30<br>Leu<br>Glu<br>Glu<br>Glu<br>Ala<br>110 | 15<br>Ser<br>Lys<br>Asp<br>Ala<br>Ser<br>95<br>Pro | Trp Gln Gln Phe 80 Ile Thr                   |   |   |     |
| 300<br>301<br>302<br>303<br>304<br>305<br>306<br>307<br>308<br>309<br>310<br>311<br>312<br>313<br>314<br>315<br>316                                    | <pre>&lt;400&gt; Met Se 1 Gly Le Val As Pro Pr 50 Asp Il 65 Asn Ar Leu Ly Arg Hi</pre>                      | SEQUE<br>T Arg<br>Tu Gln<br>In Cys<br>35<br>To Leu<br>e Leu<br>g Ala<br>s Asn<br>s Pro<br>115             | NCE:<br>Leu<br>Ala<br>20<br>Ser<br>Pro<br>Met<br>Val<br>Leu<br>100<br>Ile               | 25<br>Pro<br>5<br>Pro<br>Asn<br>Leu<br>Glu<br>Lys<br>85<br>Leu<br>His               | Val<br>Met<br>Leu<br>Asn<br>70<br>Ser<br>Pro               | Leu Thr Ile Asp 55 Asn Leu Cys Lys           | Leu Gln Asp 40 Phe Leu Gln Leu Asp 120       | Leu Thr 25 Glu Asn Arg Asn Pro 105 Gly | 10<br>Thr<br>Ile<br>Asn<br>Arg<br>Ala<br>90<br>Leu<br>Asp | Pro Ile Leu Pro 75 Ser Ala Trp        | Leu Thr Asn 60 Asn Ala Thr                   | Lys His 45 Gly Leu Ile Ala Glu 125 | Thr<br>30<br>Leu<br>Glu<br>Glu<br>Ala<br>110<br>Phe | 15<br>Ser<br>Lys<br>Asp<br>Ala<br>Ser<br>95<br>Pro | Trp<br>Gln<br>Gln<br>Phe<br>80<br>Ile<br>Thr |   |   |     |
| 300<br>301<br>302<br>303<br>304<br>305<br>306<br>307<br>308<br>309<br>310<br>311<br>312<br>313<br>314<br>315<br>316<br>317                             | <pre>&lt;400&gt; Met Se 1 Gly Le Val As Pro Pr 50 Asp Il 65 Asn Ar Leu Ly Arg Hi Lys Le</pre>               | SEQUE<br>T Arg<br>Tu Gln<br>Th Cys<br>35<br>To Leu<br>E Leu<br>Ty Ala<br>Ts Asn<br>S Pro<br>115<br>Tu Thr | NCE:<br>Leu<br>Ala<br>20<br>Ser<br>Pro<br>Met<br>Val<br>Leu<br>100<br>Ile               | 25<br>Pro<br>5<br>Pro<br>Asn<br>Leu<br>Glu<br>Lys<br>85<br>Leu<br>His               | Val<br>Met<br>Leu<br>Asn<br>70<br>Ser<br>Pro               | Leu Thr Ile Asp 55 Asn Leu Cys Lys           | Leu Gln Asp 40 Phe Leu Gln Leu Asp 120       | Leu Thr 25 Glu Asn Arg Asn Pro 105 Gly | 10<br>Thr<br>Ile<br>Asn<br>Arg<br>Ala<br>90<br>Leu<br>Asp | Pro Ile Leu Pro 75 Ser Ala Trp        | Leu Thr Asn 60 Asn Ala Thr Asn Ala           | Lys His 45 Gly Leu Ile Ala Glu 125 | Thr<br>30<br>Leu<br>Glu<br>Glu<br>Ala<br>110<br>Phe | 15<br>Ser<br>Lys<br>Asp<br>Ala<br>Ser<br>95<br>Pro | Trp<br>Gln<br>Gln<br>Phe<br>80<br>Ile<br>Thr |   |   |     |
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| 300<br>301<br>302<br>303<br>304<br>305<br>306<br>307<br>308<br>309<br>310<br>311<br>312<br>313<br>314<br>315<br>316<br>317<br>318<br>319               | <pre>&lt;400&gt; Met Se 1 Gly Le Val As Pro Pr 50 Asp II 65 Asn Ar Leu Ly Arg Hi Lys Le 13 Thr Th</pre>     | SEQUE<br>T Arg<br>Tu Gln<br>Th Cys<br>35<br>To Leu<br>E Leu<br>Ty Ala<br>S Asn<br>S Pro<br>115<br>U Thr   | NCE:<br>Leu<br>Ala<br>20<br>Ser<br>Pro<br>Met<br>Val<br>Leu<br>100<br>Ile<br>Phe        | 25<br>Pro<br>5<br>Pro<br>Asn<br>Leu<br>Glu<br>Lys<br>85<br>Leu<br>His               | Val<br>Met<br>Leu<br>Asn<br>70<br>Ser<br>Pro<br>Ile<br>Leu | Leu Thr Ile Asp 55 Asn Leu Cys Lys Lys 135   | Leu Gln Asp 40 Phe Leu Gln Leu Asp 120 Thr   | Leu Thr 25 Glu Asn Arg Asn Pro 105 Gly | 10<br>Thr<br>Ile<br>Asn<br>Arg<br>Ala<br>90<br>Leu<br>Asp | Pro Ile Leu Pro 75 Ser Ala Trp        | Leu Thr Asn 60 Asn Ala Thr Asn Ala           | Lys His 45 Gly Leu Ile Ala Glu 125 | Thr<br>30<br>Leu<br>Glu<br>Glu<br>Ala<br>110<br>Phe | 15<br>Ser<br>Lys<br>Asp<br>Ala<br>Ser<br>95<br>Pro | Trp<br>Gln<br>Gln<br>Phe<br>80<br>Ile<br>Thr |   |   |     |
| 300<br>301<br>302<br>303<br>304<br>305<br>306<br>307<br>308<br>309<br>310<br>311<br>312<br>313<br>314<br>315<br>316<br>317<br>318<br>319<br>320        | <pre>&lt;400&gt; Met Se 1 Gly Le Val As Pro Pr 50 Asp II 65 Asn Ar Leu Ly Arg Hi Lys Le 13 Thr Th 145</pre> | SEQUE<br>T Arg  | NCE:<br>Leu<br>Ala<br>20<br>Ser<br>Pro<br>Met<br>Val<br>Leu<br>100<br>Ile<br>Phe<br>Ser | 25<br>Pro<br>5<br>Pro<br>Asn<br>Leu<br>Glu<br>Lys<br>85<br>Leu<br>His<br>Tyr        | Val<br>Met<br>Leu<br>Asn<br>70<br>Ser<br>Pro<br>Ile<br>Leu | Leu Thr Ile Asp 55 Asn Leu Cys Lys Lys 135   | Leu Gln Asp 40 Phe Leu Gln Leu Asp 120 Thr   | Leu Thr 25 Glu Asn Arg Asn Pro 105 Gly | 10<br>Thr<br>Ile<br>Asn<br>Arg<br>Ala<br>90<br>Leu<br>Asp | Pro Ile Leu Pro 75 Ser Ala Trp        | Leu Thr Asn 60 Asn Ala Thr Asn Ala           | Lys His 45 Gly Leu Ile Ala Glu 125 | Thr<br>30<br>Leu<br>Glu<br>Glu<br>Ala<br>110<br>Phe | 15<br>Ser<br>Lys<br>Asp<br>Ala<br>Ser<br>95<br>Pro | Trp<br>Gln<br>Gln<br>Phe<br>80<br>Ile<br>Thr |   |   |     |
| 300<br>301<br>302<br>303<br>304<br>305<br>306<br>307<br>308<br>309<br>310<br>311<br>312<br>313<br>314<br>315<br>316<br>317<br>318<br>319<br>320<br>322 | <pre>&lt;400&gt; Met Se 1 Gly Le Val As Pro Pr 50 Asp II 65 Asn Ar Leu Ly Arg Hi Lys Le 13 Thr Th</pre>     | SEQUEER Arg   | NCE: Leu Ala 20 Ser Pro Met Val Leu 100 Ile Phe Ser                                     | 25<br>Pro<br>5<br>Pro<br>Asn<br>Leu<br>Glu<br>Lys<br>85<br>Leu<br>His<br>Tyr<br>Leu | Val<br>Met<br>Leu<br>Asn<br>70<br>Ser<br>Pro<br>Ile<br>Leu | Leu Thr Ile Asp 55 Asn Leu Cys Lys Lys 135   | Leu Gln Asp 40 Phe Leu Gln Leu Asp 120 Thr   | Leu Thr 25 Glu Asn Arg Asn Pro 105 Gly | 10<br>Thr<br>Ile<br>Asn<br>Arg<br>Ala<br>90<br>Leu<br>Asp | Pro Ile Leu Pro 75 Ser Ala Trp        | Leu Thr Asn 60 Asn Ala Thr Asn Ala           | Lys His 45 Gly Leu Ile Ala Glu 125 | Thr<br>30<br>Leu<br>Glu<br>Glu<br>Ala<br>110<br>Phe | 15<br>Ser<br>Lys<br>Asp<br>Ala<br>Ser<br>95<br>Pro | Trp<br>Gln<br>Gln<br>Phe<br>80<br>Ile<br>Thr |   |   |     |

VERIFICATION SUMMARY

DATE: 07/10/2002

PATENT APPLICATION: US/09/975,132A

TIME: 10:51:55

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Output Set: N:\CRF3\07102002\1975132A.raw